Cardiovascular performance with *E. coli* challenges in a canine model of human sepsis

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NATANSON, CHARLES, ROBERT L. DANNER, MITCHELL P. FINK, THOMAS J. MACVITTIE, RICHARD I. WALKER, JAMES J. CONKLIN, AND JOSEPH E. PARRILLO. *Cardiovascular performance with E. coli challenges in a canine model of human sepsis.* Am. J. Physiol. 254 (Heart Circ. Physiol. 23): H558–H569, 1988.—We investigated cardiovascular dysfunction by injecting lethal and nonlethal bacterial challenges into conscious dogs. *E. coli* bacteria of varying numbers were placed in a peritoneal clot. Cardiovascular function was studied with simultaneous radionuclide scans and thermodilution cardiac outputs. In surviving animals, the number of bacteria in the clot increased as the corresponding systolic cardiac function decreased ($P = 0.01$). Cardiac function was measured by left ventricular (LV) ejection fraction (EF) and LV function curves [LV stroke work index (LVSWI) vs. end-diastolic volume index (EDVI), and peak systolic pressure vs. end-systolic volume index]. Furthermore, the diastolic volume-pressure relationship of survivors shifted progressively to the right [i.e., increasing EDVI ($P < 0.02$) with minimal change (P = NS) in LV filling pressure]. This increase in LV size was associated with maintenance of measures of cardiac performance [stroke volume index (SVI) and stroke work index (SWI)] at similar levels. Death occurred only in the group with the highest bacterial dose. Compared with survivors receiving the same number of bacteria, nonsurvivors had a decrease in ($P < 0.05$) LV size, a leftward shift ($P < 0.01$) in LV diastolic volume-pressure relationship, and a decrease in both LVSWI and SVI (possibly related to volume and/or LV functional status). Data from survivors suggest that increasing the number of bacteria produces changes in myocardial compliance and contractility. These changes increase LV size (preload), a major determinant of cardiac performance that possibly enhances survival.

conscious dogs; heart function; bacterial shock

PREVIOUS STUDIES of septic shock in animal models used a number of techniques to study changes in cardiovascular function at 1–12 h after onset of the disease (1, 2, 11, 12, 14–17, 19, 31, 32). Most of these investigations demonstrated evidence of acute myocardial depression, whereas other studies demonstrated that early myocardial changes relate to dose of the challenge agent (bacteria or endotoxin) (4, 10, 20).

Recent studies in human septic shock demonstrated that cardiovascular function continues to change over several days (7, 24). These studies showed that over 2–3 days following onset of hypotension left ventricular ejection factor (LVEF) fell to a nadir associated with left ventricular (LV) dilatation and maintenance of normal or increased cardiac output. In survivors, these cardiovascular changes returned to normal over 7–10 days.

We recently described a canine model of septic shock that simulates the serial changes in cardiovascular function of human septic shock (21). In that study, animals developed a decrease in LVEF and LV dilatation, but maintained normal or high cardiac index (with volume administration) 2–3 days after a bacterial clot was placed in the peritoneum (3, 21). Further analysis of hemodynamic data of these dogs demonstrated a reversible diastolic cardiac abnormality. At 2–3 days after onset of septic shock, LV dilatation occurred with no increase in pressure, suggesting an increase in LV compliance. These cardiac diastolic and systolic abnormalities returned to near normal in 7–10 days, a pattern similar to that reported previously in human septic shock (7, 21, 24).

In the present study, a fibrin clot of increasingly lethal doses of viable *E. coli* was placed into the peritoneal cavity of dogs. Comparison between lethal and nonlethal bacterial challenges helped to clarify differences between compensated and decompensated cardiovascular response to severe septic toxicity. These observations have important implications for the understanding of human septic shock.

METHODS

Experimental design. The protocol for this study is based on methods previously described (Fig. 1) (21). We placed femoral and pulmonary arterial catheters in awake, nonsedated animals, using only local infiltration anesthesia with 1% lidocaine. Hemodynamic data were obtained simultaneously from conscious, nonsedated dogs with both intravascular catheters and gated radionuclide cineangiograms of the LV. Animals then received a volume infusion (lactated Ringer solution, 80 ml/kg body wt over 30 min) after which complete hemodynamic and radionuclide studies were repeated. All intravascular catheters were placed and removed on the same day as the comprehensive evaluation. This evaluation, which included routine laboratory tests (arterial blood gas, com-

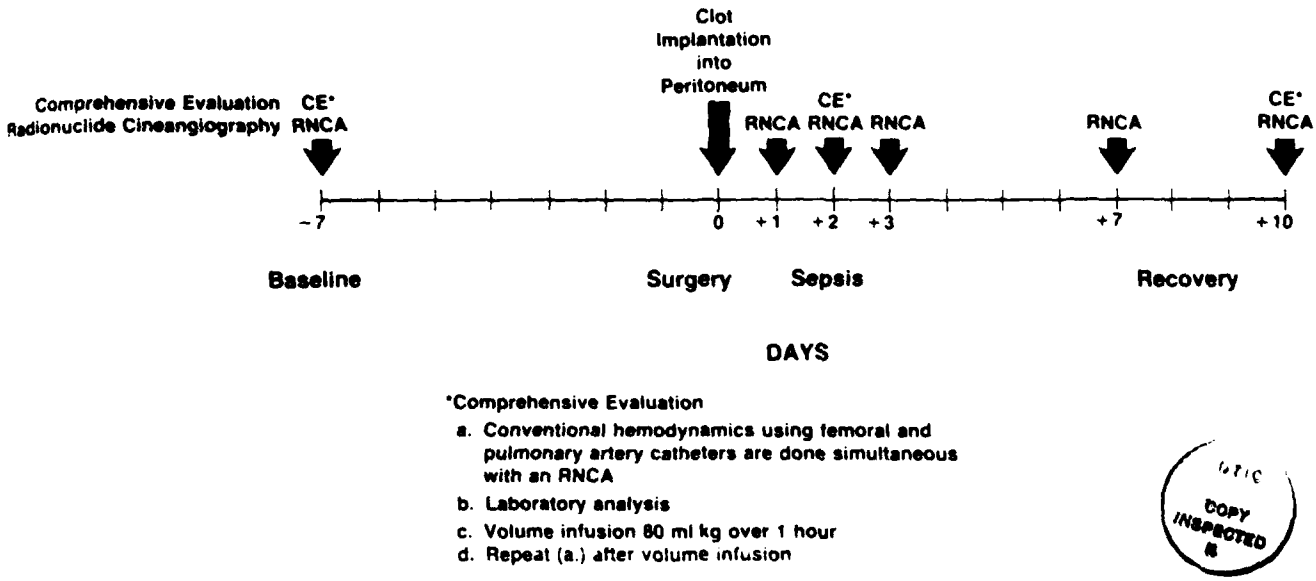

FIG. 1. Outline of experimental design and time course in 4 groups of dogs. Three groups of dogs received increasing numbers of *E. coli* in a clot placed in the peritoneum. Controls received a sterile clot. This figure was modified from Natanson et al. (*J. Clin. Invest.* 78: 260, 1986) and was included for clarification of methods used in this series of studies.

plete blood count, sodium, potassium, chloride, bicarbonate, calcium, glucose, blood urea nitrogen, and creatinine), was obtained at three time points (Fig. 1): 7 days before surgery, *day 2*, and 7–10 days postsurgery.

On *day 0*, a fibrin clot was placed surgically into each animal. The clot was either sterile for control animals or infected with one of three doses of *E. coli*: 7, 14, or 30 × 10^9 organisms/kg body wt (Table 1). Preoperatively, animals received nothing by mouth for 12 h. At all other times, the animals had unrestricted access to water and food. Nuclear heart scans were also done on *days 1* and *3* postsurgery to evaluate serial changes in EF.

On *day 1*, the animals receiving 14 × 10^9 organisms/kg body wt and 8 of the 14 control dogs [reported previously (21)] were evaluated comprehensively for early hemodynamic effects of sepsis. No serial differences in hemodynamics were found in any dogs receiving or not receiving volume infusion; however, comparison was made only between animals receiving similar treatment at each time point.

Surgical procedures and clot preparation. Fifty-three 2-year-old male beagles (weighing 11.40 ± 0.21 kg; means ± SE) were used. The method used for surgical implantation of sterile or infected clot into the peritoneum is described previously by Fink et al. (8) and Natanson et al. (21). Dose of bacteria implanted in 39 dogs, and number of dogs receiving each challenge are shown in Table 1.

TABLE 1. *Bacterial dose, day of demise, and number of survivors and nonsurvivors*

	Bacterial Implant, no./kg body wt			
	0	7 × 10^9	14 × 10^9	30 × 10^9
n	14	8	10	21
Deaths by specific day				
0–1	0	0	0	6
1–2	0	0	0	3
2–3	0	0	0	3
3–5	0	0	0	1
Total deaths	0	0	0	13
Total survivors	14	8	10	8
Percent mortality	0	0	0	62

n, No. of dogs in each bacterial dose group.

Physiological measurements and hemodynamic calculations. All measurements were made, by techniques previously described (21), from indwelling femoral and balloon flotation pulmonary artery catheters in nonanesthetized animals resting quietly in slings. Radionuclide-gated blood-pool scanning was performed with conventional techniques (21). Hemodynamic data were indexed by body weight in kilograms. The following data were calculated according to standard formulas (21): left ventricular stroke work index (LVSWI), $g \cdot m \cdot kg^{-1}$; systemic vascular resistance index (SVRI), $dyn \cdot s \cdot cm^{-5} \cdot kg^{-1}$; cardiac index (CI), $ml \cdot kg^{-1} \cdot min^{-1}$; and stroke volume index (SVI), $ml \cdot kg^{-1} \cdot beat^{-1}$. The end-diastolic volume index (EDVI) (ml/kg) and end-systolic volume index (ESVI) (ml/kg) were calculated from hemodynamic studies and radionuclide scans obtained simultaneously. The formulas EDVI = SVI (from thermodilution cardiac output)/EF (from radionuclide cineangiography) and ESVI = EDVI − SVI were used to obtain these calculations.

Statistical methods. Pairs of mean values were compared by Student's *t* test and several mean values by *F* test. A Bartholomew test was used to compare differences among ordered means (22), and the Dunnett test was used to compare multiple experimental means with the corresponding control (6). Linear regressions were compared using the method of least squares.

To simplify comparison of serial hemodynamics changes among different groups of dogs (Figs. 2, 5, and 6), we demonstrated these changes from a common origin

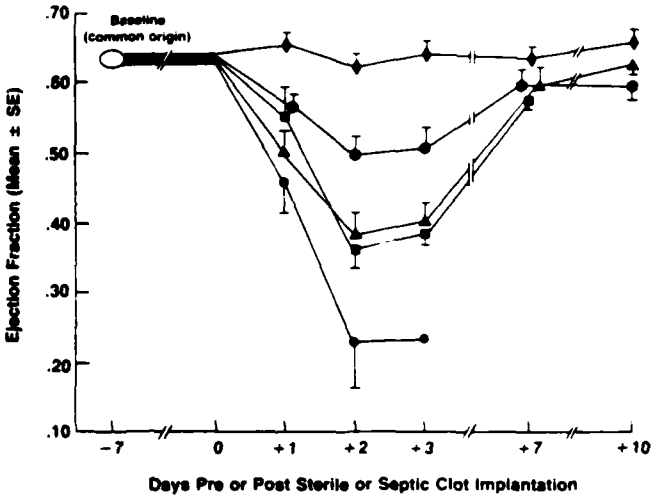

FIG. 2. Serial ejection fractions (EFs) in infected and control dog groups as a function of time in days. EF (means ± SE) for different bacterial dosage groups of dogs are represented by controls (♦), 7×10^9 (●), 14×10^9 (▲), 30×10^9 survivors (■), and 30×10^9 nonsurvivors (●). No. of dogs in each group and no. of survivors are shown in Table 1. Common origin is shown by ○ representing mean EF for all dogs (infected and control) at *day* −7 (base line). This is a presepsis time point before any intervention. Serial changes in LVEF are graphed for ease of comparison of different groups from this base line of common origin (see METHODS).

(base line). To justify this procedure, we applied an F test to the base-line mean values of the various groups of animals accepting the null hypothesis only if $P > 0.20$.

The changes in EDVI and in pulmonary capillary wedge pressure (PCWP) were computed as previously described by subtracting the preinfusion value from the corresponding postinfusion value for each dog and then dividing in half (Fig. 7) (21). This value was then added to the respective preinfusion value to obtain the coordinates of the midpoint of the arrow denoting the change during volume infusion. The respective coordinates were then averaged over appropriate groups of dogs and are shown as black rectangles.

The well-known relationship, cardiac index = heart rate × stroke volume index, was used to compare the relative contributions of heart rate (HR) and SVI with the change in cardiac index caused by volume loading. Let $CI_1 = HR_1 \times SVI_1$ be the version of the above relation observed before volume loading and $CI_2 = HR_2 \times SVI_2$ be the version seen after loading. The relative increase in CI can be expressed as the product of the respective increases in HR and SVI by dividing the latter equation by the former, namely

$$\frac{CI_2}{CI_1} = \frac{HR_2}{HR_1} \times \frac{SVI_2}{SVI_1}$$

Comparison of the relative changes in HR and in SVI is in effect a comparison of the corresponding relative contributions of changes in HR and SVI to the relative increase in CI. Since it is easier to compare differences rather than ratios, the necessary comparisons were carried out in log scale; accordingly logs of the relevant data were taken and then averaged over days (Fig. 9, *A* and *B*).

RESULTS

Blood cultures and clinical manifestations. Two days after surgery, all surviving dogs implanted with an infected clot had positive blood cultures. In all dogs, organisms grown from blood cultures were species specific for the implanted microorganism; blood cultures were sterile at base line and recovery. Three days after surgery, all bacteremic dogs were febrile, weak, and lethargic. Animals receiving a higher dose of bacteria were more seriously ill. Despite no antibiotic treatment, pressor therapy, or intervention other than volume infusion, all 14 control animals and 26 of the 39 infected animals clinically recovered within 7–10 days after surgery. Only 13 animals died out of a total of 21 that received the highest dose of bacteria (30×10^9). Six animals died within 24 h after clot implantation; 3 between 24 and 48 h; 3 between 48 and 72 h; and 1 after 72 h (Table 1). All 14 control dogs implanted with a sterile clot were afebrile. These dogs had negative blood cultures and appeared healthy throughout the study.

Serial EFs with increasing doses of bacteria. Serial mean (±SE) changes in EFs before volume infusion were studied both in infected and control dogs (Fig. 2). As the dose of bacteria increased, mean EF decreased during *days 1–2* following the onset of septic shock.

To compare changes in EF with different bacterial doses, the mean maximum decrease in EF (compared with base line) was determined. The LVEF (maximum) decreased as the dose increased ($0 > 7 > 14 > 30 \times 10^9$ survivors $> 30 \times 10^9$ nonsurvivors, $P \doteq 0.01$, Bartholomew test). Mean change in EF from base line to recovery among survivors in all four groups of dogs was not statistically different ($P > 0.20$) from 0. Thus decrease in EF depended on bacterial dose. EF value returned near to normal in survivors of septic shock.

In the 26 infected survivors, maximum decrease in EF occurred on *day 2* in 22 dogs, on *day 3* in 4 dogs. Recovery occurred in 7–10 days, independent of bacterial dose. In the 13 nonsurvivors (30×10^9), maximum decrease in EF was observed on the last determination before death, which occurred within a few hours of surgery in six animals. In septic and control dogs, volume infusion never induced a significant change in mean EF (data not shown).

Mean shifts in EDVI. Mean changes in EDVI between base line and *day 2* prevolume and *day 2* pre- to postvolume infusion were studied for control animals and those receiving various doses of bacteria (Fig. 3). On *day 2* prior to volume infusion, mean EDVI in infected surviving dogs increased ($P < 0.05$) in response to increasing dose of bacteria ($30 \times 10^9 > 14 \times 10^9 > 7 \times 10^9$). In the group receiving the highest dose (30×10^9), survivors as compared with nonsurvivors had a higher increase ($P < 0.05$) in mean EDVI from base-line to *day 2*.

All infected dogs had a significant increase ($P < 0.05$) in mean EDVI with volume infusion on *day 2*. Response to volume infusion was similar in all three groups of infected surviving dogs ($P > 0.25$). In the group receiving the highest dose, the mean response in EDVI on *day 2* to volume loading was not statistically different in survivors as compared with nonsurvivors. In survivors, ven-

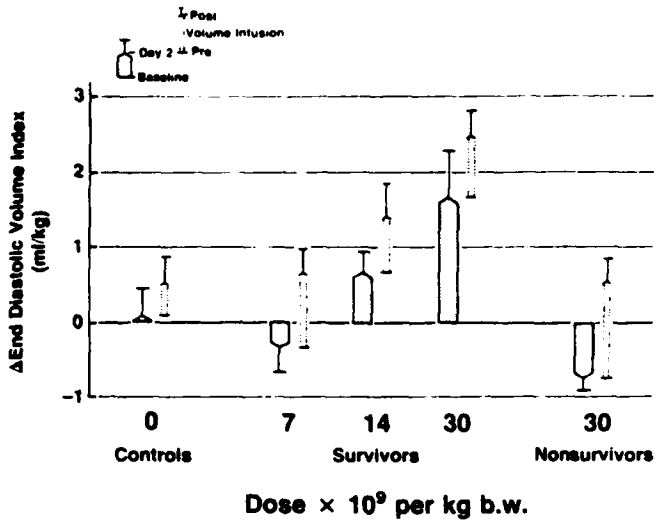

FIG. 3. Means ± SE changes in end-diastolic volume index (EDVI) in infected and control dog groups represented from base line to *day 2* with stippled arrow and *day 2* pre- to postvolume infusion with open arrow. Response to volume infusion on *day 2* was plotted in addition to change from base line to *day 2*. This was done to show response to volume on *day 2* (length of open arrow) and change from base line to *day 2* post-volume infusion (origin of stippled arrow to tip of open arrow). No. of dogs in each group and percent of survivors are shown in Table 1. In the 30 × 10^9 group, 2 survivors and 1 nonsurvivor are not included because they did not receive a volume infusion on *day 2*.

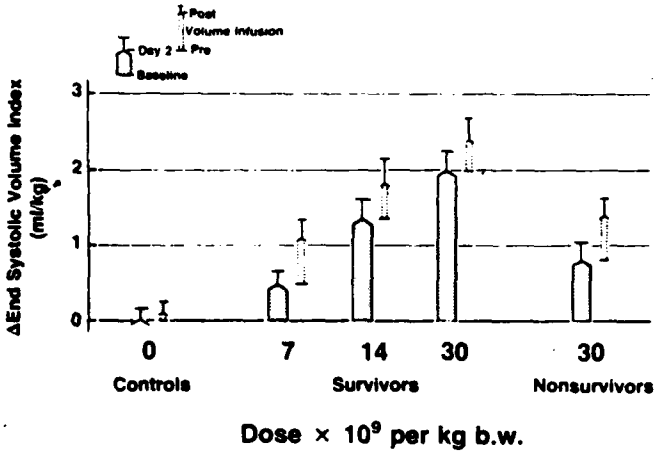

FIG. 4. Means ± SE changes in end-systolic volume index (ESVI) in infected and control dog groups. Format is same as Fig. 3.

tricular size returned close to base-line value in 7–10 days (recovery data not shown). In controls, mean EDVI on *day 2* pre- and postvolume demonstrated no significant shift from base line.

Mean shifts in ESVI. Mean change in ESVI between base line and *day 2* prevolume and *day 2* pre- to postvolume infusion was studied for each bacterial dose group and controls (Fig. 4). On *day 2* prior to volume infusion, mean ESVI in infected surviving dogs showed an increase ($P < 0.02$) that corresponded to increasing doses of bacteria. In the highest dose group, survivors had a greater increase ($P < 0.05$) than nonsurvivors in mean ESVI from base line to *day 2*.

All infected dogs had a significant increase ($P < 0.05$) in ESVI with volume infusion on *day 2*. Response to volume infusion was similar ($P > 0.10$) in all three groups of infected survivors. Mean ESVI response to volume loading on *day 2* was not significantly different among the survivors as compared with nonsurvivors in the group receiving the highest dose of bacteria. In survivors, ventricular size returned near to base-line value in 7–10 days. In controls, mean ESVI on *day 2* pre- and postvolume demonstrated no significant change from base line.

Mean shifts in Frank-Starling relationship plots. The effect of load on left ventricular performance was studied using plots to show the influence of change in ventricular filling (EDVI) during volume infusion on change in stroke work (LVSWI), Frank-Starling relationship plot (Fig. 5). Prevolume *day 2* mean LVSWI was similar ($P > 0.25$) among all three infected survivor groups and was depressed compared with that of the control group. Compared with controls, this decrease in LVSWI was only significant ($P < 0.05$) in the 7 and 14 × 10^9 groups. On *day 2*, mean value for LVSWI increased ($P < 0.01$) after volume infusion in the three groups of infected survivors. Response to volume was similar ($P > 0.25$) in the three groups, bringing the mean value for LVSWI to near the control value of prevolume *day 2*.

As dose of bacteria increased, prevolume *day 2* EDVI in the three infected survivor groups increased ($P < 0.05$) (30 × 10^9 > 14 × 10^9 > 7 × 10^9) (Fig. 3). On *day 2* after volume, mean EDVI increased further (30 × 10^9 > 14 × 10^9 > 7 × 10^9).

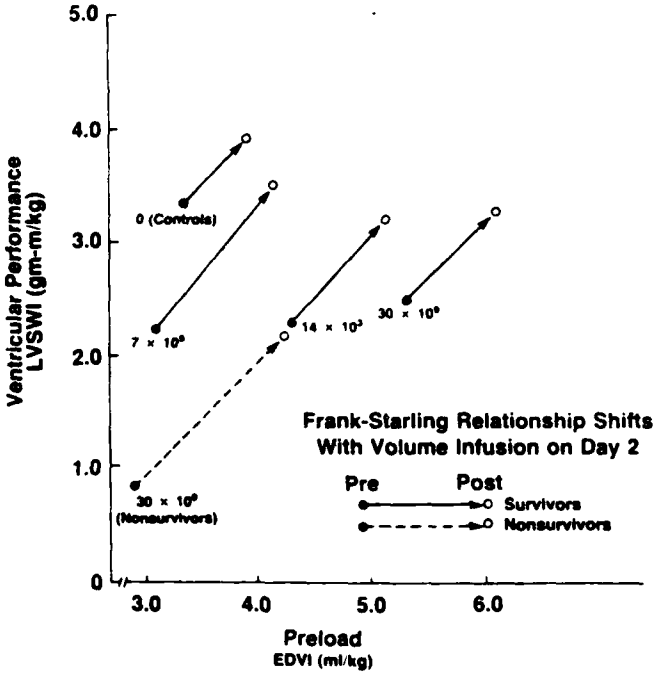

FIG. 5. Plots of mean shift in ventricular function for infected dogs and controls on Frank-Starling relationship plots of end-diastolic volume index (EDVI) vs. left ventricular stroke work index (LVSWI) on *day 2* pre- to postvolume infusion. Mean prevolume infusion values on *day 2* for EDVI vs. LVSWI are shown for each group by ● labeled with doses of bacteria per kilogram of body weight. Mean values for EDVI vs. LVSWI postvolume infusion are shown by ○. Shifts in LV function in survivors are depicted by ↗ and in nonsurvivors by ↘. In 30 × 10^9 group, 2 survivors and 1 nonsurvivor are not included for pre- and postvolume infusions, because they did not receive a volume infusion that day.

On *day 2* prevolume infusion, the highest dose group nonsurvivors as compared with survivors decreased ($P < 0.05$) mean LVSWI and EDVI. On *day 2* after volume infusion, nonsurvivors increased ($P < 0.05$) both mean EDVI and LVSWI; however, *day 2* LVSWI did not increase up to prevolume mean control value as seen in survivors (30×10^9), and EDVI did not shift further to the right beyond the 14×10^9 group (Fig. 5). By 7–10 days (recovery), ventricular function returned close to base-line value, in infected survivors both pre- and postvolume infusion (recovery data not shown).

Increasing dose of bacteria in infected survivors was associated with a shift to the right ($30 \times 10^9 > 14 \times 10^9 > 7 \times 10^9$) of a downward-shifted Frank-Starling relationship plot [i.e., the left ventricle progressively dilated (increasing in EDVI) and maintained a similarly depressed LV performance (LVSWI)]. On *day 2* the nonsurvivors as compared with survivors had a larger downward shift but a smaller rightward shift of the Frank-Starling relationship (Fig. 5).

Mean shifts in end-systolic volume-pressure relationship plots. Effects of sepsis on left ventricular performance were further studied by examining the relationship with volume loading between end-systolic volume index and peak systolic pressure (PSP) (Fig. 6). The evaluation was performed on *day 2*, the day of maximum decrease in ventricular performance. Prevolume *day 2* mean PSP was similar ($P > 0.10$) among the infected survivor groups and was depressed ($P < 0.01$) compared with that of controls. On *day 2* after volume infusion, mean value for PSP increased ($P < 0.01$) in infected survivors. Response to volume was not statistically different (P = NS) for the three groups, bringing mean value for PSP close to prevolume *day 2* control value.

Prevolume *day 2* ESVI in the three infected survivor groups increased ($P < 0.02$) as the dose of bacteria increased (Fig. 4). On *day 2* after volume, mean ESVI further increased ($30 \times 10^9 > 14 \times 10^9 > 7 \times 10^9$).

Mean value for PSP and ESVI (prevolume infusion) in nonsurvivors, as compared with survivors of the highest dose group (30×10^9) decreased, but this decrease was only significant ($P < 0.05$) for ESVI. On *day 2* after volume infusion, nonsurvivors increased ($P < 0.05$) PSP and ESVI. In survivors, both pre- and postvolume infusion, ventricular function returned near to base-line value in 7–10 days (recovery data not shown).

Comparing *day 2* to base line in surviving dogs, increasing dose of bacteria was associated with movement to the right ($30 \times 10^9 > 14 \times 10^9 > 7 \times 10^9$) of a downward-shifted end-systolic volume-pressure plot. Compared with survivors (30×10^9), nonsurvivors had an end-systolic volume-pressure plot that shifted downward to the left (although not significantly).

Mean shift in relationship between EDVI and PCWP. Effect of increasing dose of bacteria on the relationship between mean EDVI and mean PCWP was examined, both pre- and postvolume infusion from base line to *day 2* (Fig. 7). To examine whether the dose of bacteria correlates to shifts in volume-pressure relationship (compliance) between base line and *day 2*, midpoint value was calculated for EDVI and PCWP from pre- to postvolume infusion points for each dog on each day and averaged over each group of dogs (see METHODS). This method of evaluation allowed information from each pre- and postvolume infusion (a volume-pressure line) to be used in the determination of magnitude and direction of change in diastolic relationships (if any) between days.

In infected survivors, mean midpoint in EDVI increased ($P < 0.02$) with each higher dose of bacteria ($30 \times 10^9 > 14 \times 10^9 > 7 \times 10^9$). There was no difference (P = NS) in minimal change in mean midpoint PCWP among the three groups of survivors. Thus infected survivors, with an increasing dose of bacteria, had larger ventricular volume with no increase in pressure (compatible with an increase in compliance). Consequently, Fig. 7 shows a progressive shift of the volume-pressure

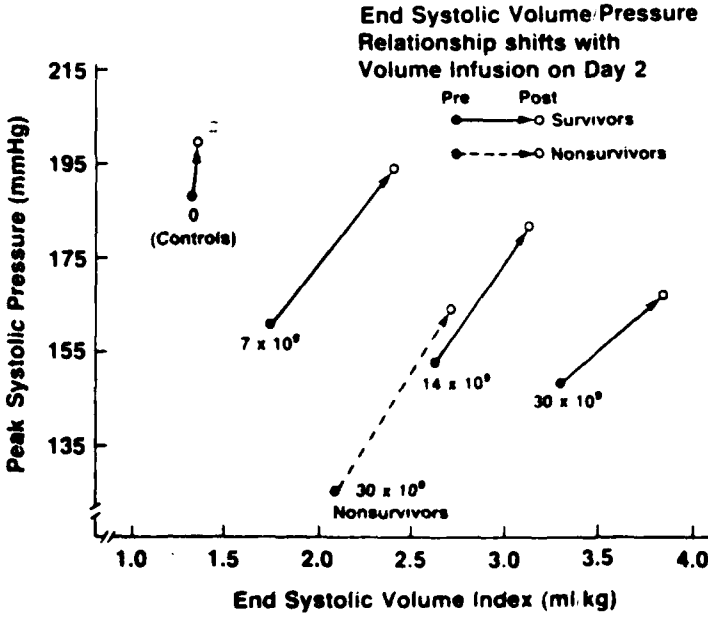

FIG. 6. Plots of mean shift in ventricular function for infected dogs and controls on end-systolic volume-pressure plots of end-systolic volume index vs. peak systolic pressure (PSP). Format is same as Fig. 5.

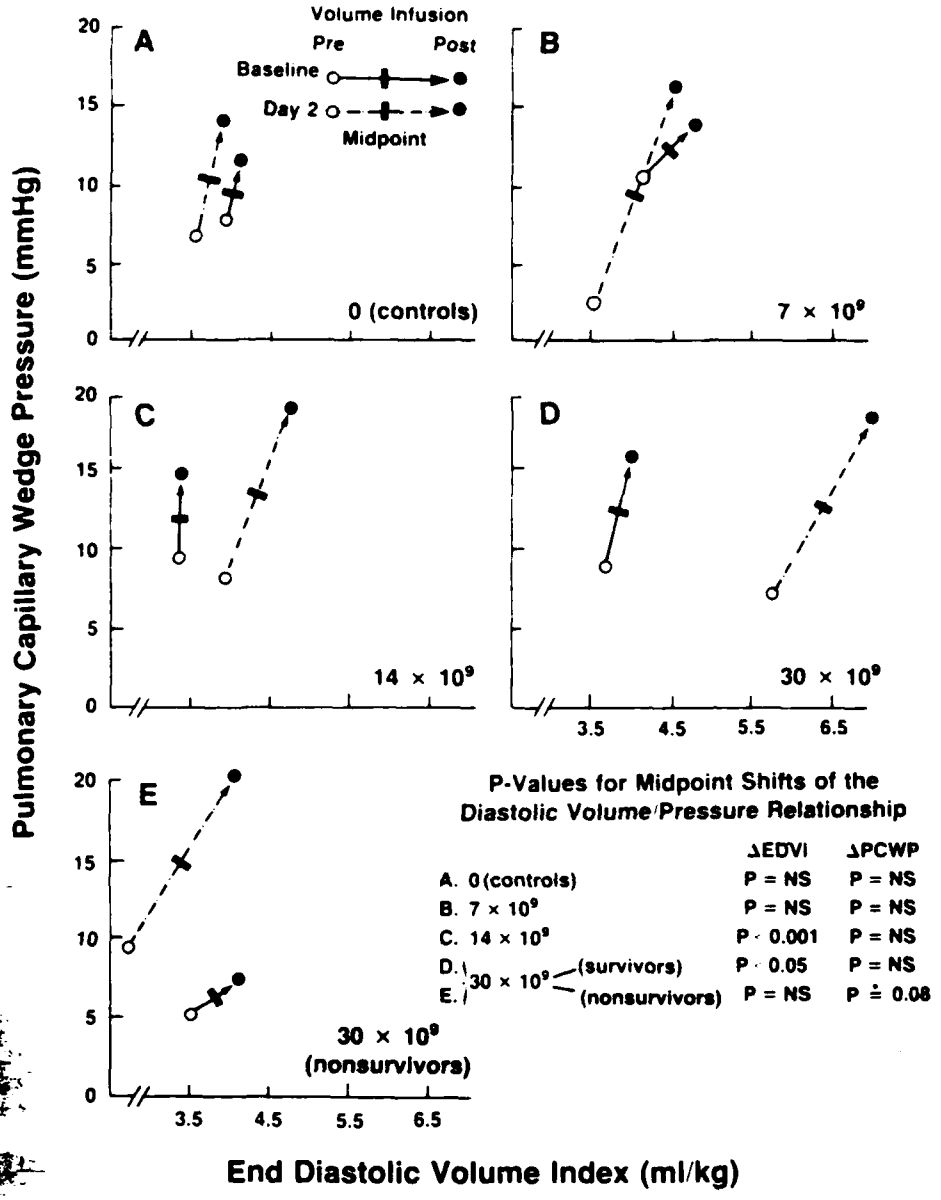

FIG. 7. Mean shifts in ventricular volume-pressure relationships, in response to volume infusion, on compliance curve plots of end-diastolic volume index (EDVI) vs. pulmonary capillary wedge pressure (PCWP). ○, Prevolume infusion values; ●, postvolume infusion values; pre- to postvolume infusion values are connected by — at base line and - - → at *day 2*; ▮, mean midpoint each day of pre- to postvolume infusion volume-pressure relationship (see METHODS). Shifts in volume-pressure relationship between days are represented by distance between ▮. The *P* values are obtained from paired-sample *t* tests comparing midpoint shift in PCWP and EDVI between base line and *day 2*. No. of dogs in each group and percent of survival are shown in Table 1. Three dogs in *A*, 4 dogs in *D*, and 1 dog in *E* are not included at base line and *day 2* pre- or postvolume infusions because they did not receive a volume infusion at base line and/or *day 2*.

relationship to the right in survivors.

Nonsurvivors, as compared with survivors (30×10^9), had a decrease ($P < 0.01$) in EDVI, with an increase in PCWP (although not significant) consistent with a decrease in compliance. This is represented in Fig. 7*E* by a shift of mean midpoint in the opposite direction. In survivors, the volume-pressure relationship returned near base-line values in 7–10 days (recovery data not shown). Controls demonstrated no significant change in mean EDVI and PCWP from base line to *day 2*.

CI, SVI, and HR. Mean change in CI, SVI, and HR between base line and *day 2* prevolume and *day 2* pre- to postvolume infusion was studied for each bacterial dose group and controls (Fig. 8, *A–C*).

Among the three infected survivor groups (7, 14, and 30×10^9) and control group, change in mean CI from base line to *day 2* showed no significant trend or statistical difference. Within the highest bacterial dose group from base line to *day 2*, nonsurvivors as compared with survivors had a greater decrease ($P < 0.05$) in mean CI.

On *day 2*, after volume loading, mean CI of all groups increased significantly ($P < 0.01$) to near or above base-line value. In the three infected survivor and control groups, mean CI response to volume loading showed no significant difference on *day 2*. Within the highest bacterial dose group (30×10^9), nonsurvivors as compared with survivors on *day 2* had a greater increase ($P < 0.05$) in mean CI with volume infusion.

Among the three infected survivor and control groups, change in mean SVI from base line to *day 2*, showed no trend or significant difference, except the lowest dose group (7×10^9). This group, from base line to *day 2*, showed a decrease ($P < 0.05$) in mean SVI as compared with controls. Within the highest dose group (30×10^9), nonsurvivors as compared with survivors had a greater decrease ($P < 0.01$) in mean SVI from base line to *day*

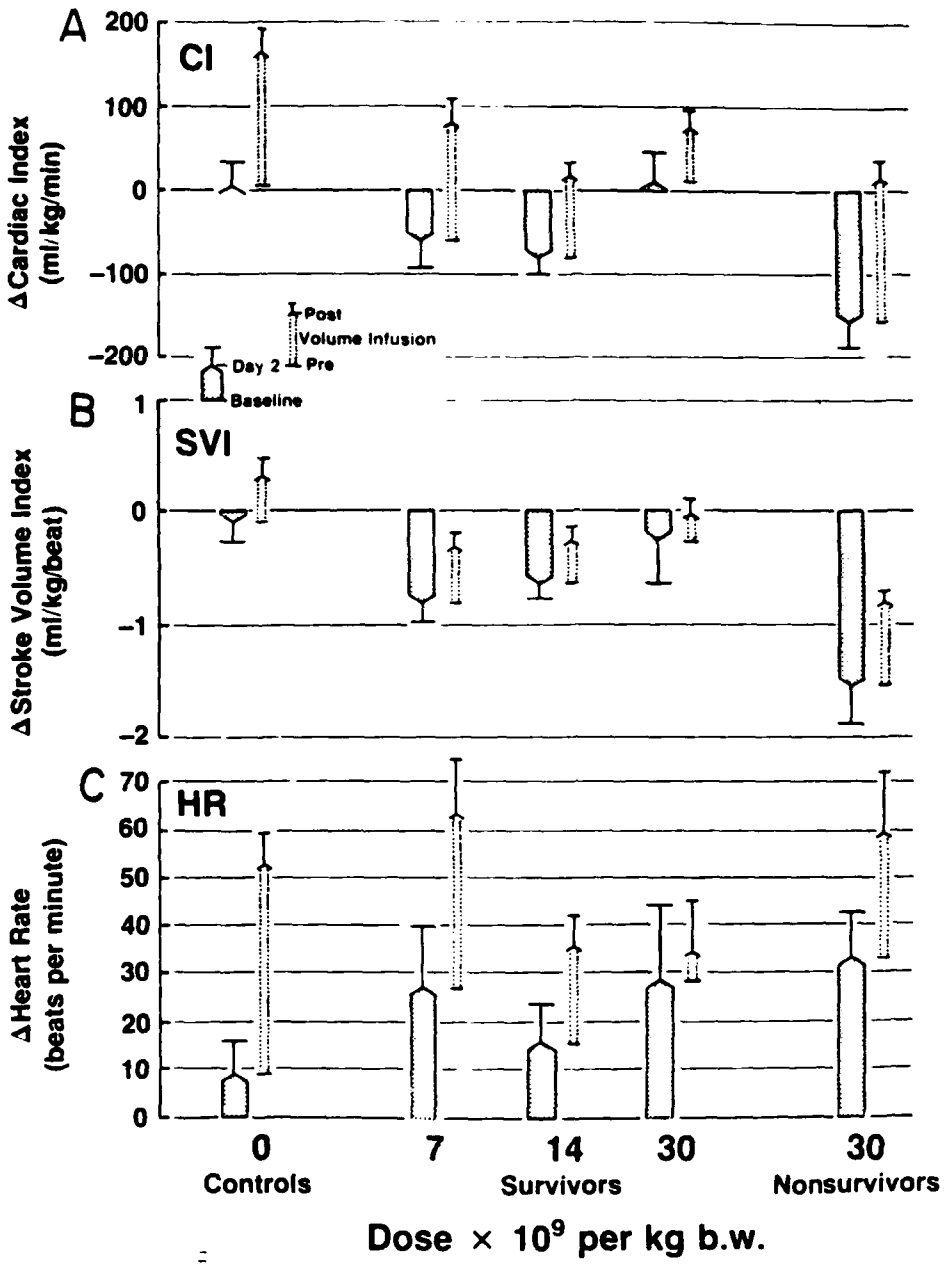

FIG. 8. Means ± SE changes in cardiac index (CI, *A*, *top*), stroke volume index (SVI, *B*, *middle*), and heart rate (HR, *C*, *bottom*) in infected and control dog groups. Format is same as Fig. 3.

2. Among infected survivors, nonsurvivors, and controls, mean SVI response on *day 2* with volume was not significantly different.

Change in mean HR from base line to *day 2* did not differ between infected groups and controls (Fig. 8*C*). Survivors with increasing dose of bacteria had a decrease ($P < 0.01$) in HR response that corresponded to volume infusion ($0 > 7 \times 10^9 > 14 \times 10^9 > 30 \times 10^9$). On *day 2*, mean HR response to volume infusion was not significantly different between nonsurvivors and survivors (30×10^9 group).

Relative changes in CI, SVI, HR, and EDVI. Mean relative increase in survivors of SVI, EDVI, CI, and HR was calculated in response to volume on *day 2* (Fig. 9). Comparison of changes in SVI and EDVI is shown in Fig. 9*A*. Fig. 9*B* demonstrates the relative contribution of HR and SVI to the CI response to volume. Since the log scale was used to plot relative increase (Fig. 9*B*), differences between HR and SVI (HR-SVI) determine which of these two parameters was the major contributor to CI response from volume infusion (see METHODS).

For survivors, the relative difference between HR and SVI decreased as dose of bacteria increased. The two groups with the highest dose (14 and 30×10^9) had a relative difference between HR and SVI that became negative (Fig. 9*B*). This indicates that an increasing dose of bacteria led to a progressively greater relative contribution from SVI and lesser contribution from HR to mean CI response seen after volume loading. In survivors, the relative contribution of mean SVI to CI increased ($P < 0.05$) with increasing number of bacteria ($0 > 7 \times 10^9 > 14 \times 10^9$), except in the highest dose group

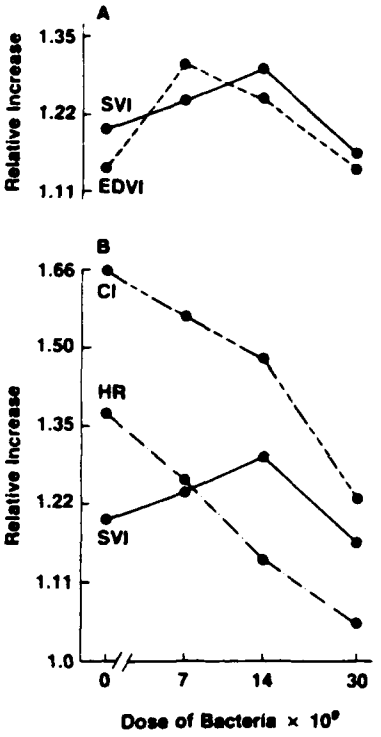

FIG. 9. Relative mean increases in response to volume on *day 2*, in end-diastolic volume index (EDVI) and stroke volume index (SVI) (*top*) and in cardiac index (CI), SVI, and heart rate (HR) (*bottom*) in infected survivors and control dog groups. Mean values for each hemodynamic parameters are connected by various pattern lines: SVI (——), EDVI (– – – –), CI (— — — —), and HR (— · —). To demonstrate relative contributions of SVI and HR to CI volume response on *day 2* by differences, log scale was used (see METHODS). Please note on *day 2* that with volume loading decreases ($P < 0.01$) in relative HR response with increasing doses of bacteria ($30 \times 10^9 > 14 \times 10^9 > 7 \times 10^9 > 0$).

(30×10^9), where SVI response showed no significant difference from controls. Therefore, progressive relative decrease in mean CI response to volume on *day 2* seen with increasing dose of bacteria (Fig. 9*B*) was predominantly due to decrease ($P < 0.01$) in mean contribution from HR response ($30 \times 10^9 > 14 \times 10^9 > 7 \times 10^9 > 0 \times 10^9$).

The progressive decline in relative mean HR response to volume on *day 2* with increasing dose of bacteria was predominantly responsible for the parallel decrease in mean CI response to volume (Fig. 9*B*). In contrast, SVI response to volume was increased or maintained, despite increasing challenge dose of bacteria. These relative changes in mean SVI paralleled the relative changes in EDVI (Fig. 9*A*).

Mean shifts in afterload and filling pressures. Mean changes in MAP, SVRI, and PCWP between base line and *day 2* prevolume and *day 2* pre- to postvolume infusion were calculated for each dog group (Fig. 10).

Changes in mean MAP from base line to *day 2* prevolume showed no significant trend in infected survivors (Fig. 10*A*). Only the groups with the two highest doses of bacteria had significant decreases ($P < 0.05$) in mean MAP as compared with controls on *day 2*. Changes in mean MAP from base line to *day 2* were not significantly different between nonsurvivors and survivors (30×10^9 group). On *day 2*, the mean MAP response to volume loading in all groups was not significantly different, except in that of the lowest dose group (7×10^9), which had a greater increase ($P < 0.05$) in mean MAP with volume loading compared with controls.

Change in mean SVRI from base line to *day 2* and in response to volume on *day 2* among survivors showed no trend or significant difference (Fig. 10*B*). In response to volume on *day 2*, all groups had a significant decrease ($P < 0.05$) in SVRI to near or below base-line values. Nonsurvivors compared with survivors within the highest bacterial dose group (30×10^9) had a greater increase ($P < 0.05$) in mean SVRI from base line to *day 2*. In response to volume on *day 2*, nonsurvivors compared with survivors had a greater decrease ($P < 0.05$) in mean SVRI.

Except for those from the lowest bacterial dose group, infected and control dogs showed no trend or difference in mean change in PCWP from base line to *day 2*. Dogs from the lowest bacterial dose group had a greater decrease ($P < 0.05$) in mean PCWP than in control dogs. There was no significant difference or trend in response to volume on *day 2* among infected and control dogs, except that those from the lowest bacterial dose group with volume had a greater increase ($P < 0.05$) in mean PCWP after volume loading than the controls.

Laboratory values. All hemodynamic studies were performed on control dogs and infected dogs with a normal pH and Po_2 (data not shown). By *day 2*, all infected dogs developed metabolic acidosis with full respiratory compensation. Level of hemoglobin, sodium, potassium, bicarbonate, chloride, glucose, and calcium were similar among infected dog groups; none of the animals developed sufficient abnormalities in these values to account for changes in LV function. Renal function (creatinine and blood urea nitrogen) remained normal throughout the study in both control and infected dogs.

In survivors and nonsurvivors of sepsis, the only significant ($P < 0.01$) laboratory abnormality was the white blood cell (WBC) count. On *day 2* of sepsis, surviving infected dogs had significant ($P < 0.01$) elevation of WBC from base-line value of 13 ± 1 to $19 \pm 1.5 \times 10^3/mm^3$; on *day 2*, nonsurvivors had a decreased ($P < 0.01$) WBC from base-line value of 14 ± 1 to $2.32 \pm 0.33 \times 10^3/mm^3$.

DISCUSSION

In infected survivors, increasing the number of bacteria at a nidus of infection was associated with both a dose-dependent, progressive decrease in systolic cardiac function and a similar, stepwise diastolic ventricular dilatation. In addition, a high bacterial dose that resulted in both survival and death produced ventricular dilatation associated with survival. These findings suggest that gram-negative sepsis produces dose-dependent changes in cardiovascular systolic and diastolic function. Such findings may lead to new information regarding the pathogenesis and cause of patient death in human septic shock.

Ventricular performance was evaluated by several different methods. Employing simultaneous measurements of thermodilution-cardiac outputs and radionuclide ci-

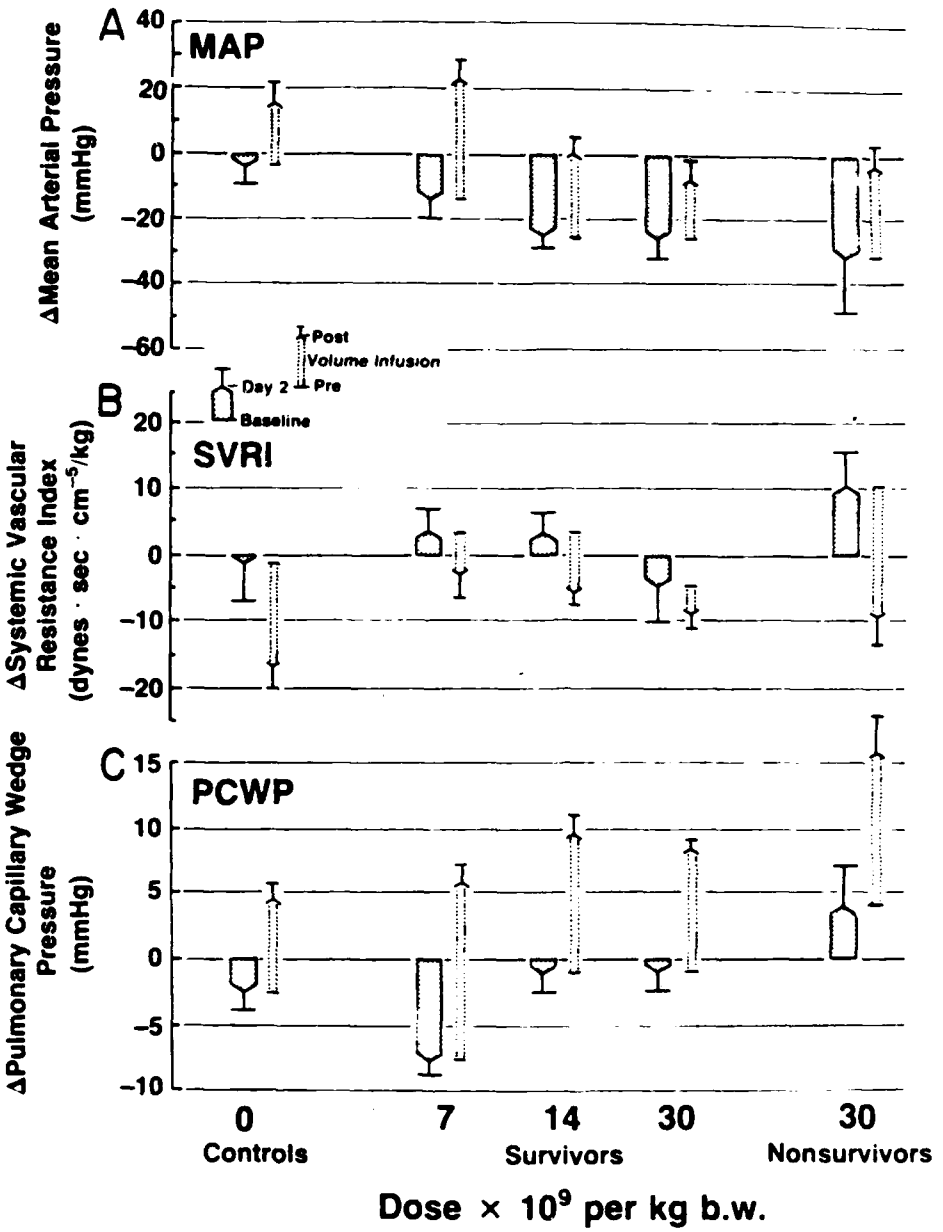

FIG. 10. Means ± SE changes in mean arterial pressure (MAP, *A*, *top*) systemic vascular resistance index (SVRI, *B*, *middle*), and pulmonary capillary wedge pressure (PCWP, *C*, *bottom*) in infected and control dog groups. Format is same as in Fig. 3.

neangiograms, we used the following methods to quantitate ventricular function in infected and control dogs: LVEF; Frank-Starling relationship with volume measurement of preload (LVSWI vs. EDVI); and, end-systolic volume-pressure plots (PSP vs. ESVI). These methods have been advocated by different groups to be excellent measures of systolic ventricular performance (9, 29). This study shows that in survivors increasing the number of bacteria placed in a peritoneal clot results in a corresponding reduction in systolic ventricular function as quantitated by these methods. Paradoxically, in survivors, certain measure of LV function (i.e., LV stroke volume and stroke work index) was maintained at similar levels, despite evidence of reduced LV contractility, as measured by these three methods of quantifying LV performance. Discrepancy between these cardiac physiological parameters is explained by the fact that during septic shock there is LV dilatation, and thus maintenance of cardiac performance via the Frank-Starling mechanism.

During septic shock, these LV changes in infected survivors cannot be explained by changes in afterload. In this study, there was no significant differences or trend in afterload, as measured by mean SVRI or MAP (pre- or postvolume infusion). Infected survivors showed a greater decrease (although not significant) in mean MAP that corresponded to increasing dose of bacteria. However, a decrease in MAP is unlikely to explain this cardiovascular change, because such a change should cause an increase in EF (decrease in LV volume) (i.e., in infected survivors, an opposite effect occurred during septic shock).

Surviving dogs receiving larger doses of bacteria had correspondingly larger LV (Figs. 3 and 4). This change

in LV size did not appear to be related to a difference in circulating volume. In general, dogs receiving the highest dose of bacteria should be the sickest and therefore should be the least likely to take liquids by mouth. These dogs should also have the most severe peritonitis with the largest third space fluid loss (i.e., such animals should have lowest circulating volume and smallest LV). We found, however, that among infected survivors, those receiving the highest dose of bacteria produced the largest increase in LV size. In infected survivors, complete replacement of intravascular volume (80 ml/kg body wt in 30 min) should have reduced any difference in LV size and function that occurred due to volume depletion. To the contrary, septic survivors had a remarkably similar increase in EDVI after fluid loading, and these animals maintained the same relationships in LV size and LV function plots (pre- and postvolume infusion, Figs. 3–6). In addition, among infected surviving dog groups there was no difference in hematocrit or blood urea nitrogen to suggest any difference in circulating volume during septic shock.

In this study, a load-sensitive measure (Frank-Starling plots and EF) and a load-insensitive measure (end-systolic volume-pressure plots) were used to quantitate LV contractility. In Frank-Starling LV function plots (Fig. 5), volume (EDVI) instead of pressure (PCWP) was used to measure preload. Volume more accurately reflects LV stretch (preload) because it is independent of sepsis-induced and volume-administration-induced compliance changes. Furthermore, EDVI was used to measure preload because recent data (9) have demonstrated that the Frank-Starling relationship between EDVI and LVSWI was linear and load independent. Thus two-point plots in Fig. 5 of EDVI vs. LVSWI reflect more complete linear Frank-Starling LV function plot. These Frank-Starling plots demonstrate that during septic shock there was a decrease in LV performance with an increase in preload (i.e., LV dysfunction).

In end-systolic volume-pressure plots (Fig. 6), peak systolic pressure was used to measure systolic pressure because it reportedly has excellent correlation with end-systolic pressure (18). Other investigators have demonstrated that a decrease in the peak systolic pressure to end-systolic volume ratio was a sensitive and specific index of systolic LV function (23, 30).

In this study, the end-systolic volume-pressure plots demonstrated that during septic shock there was a decrease in peak systolic pressure with an increase in ESVI in infected dogs, i.e., a decrease in contractility. Furthermore, in infected survivors, ESVI increased progressively at a similarly depressed peak systolic pressure (prevolume infusion). During septic shock, infected survivors with increasing dose of bacteria had a downward-shifted end-systolic volume-pressure relationship that moved progressively to the right, thus documenting a progressive decrease in LV contractility. Thus multiple indices to evaluate LV function, both preload sensitive and insensitive (EF, Frank-Starling plots, and end-systolic pressure-volume plots) documented progressive decreases in LV contractility during septic shock.

To further examine LV function in survivors, we analyzed relative changes in cardiac performance as they responded to standard volume infusion. In infected survivors with increasing dose of bacteria, there was a progressive relative decrease in CI and HR response to volume loading. However, in response to volume, the ability to increase or maintain SVI was preserved. This ability paralleled changes in LV size with volume infusion. In response to volume, with increasing dose of bacteria, survivors depended on increase or maintenance of ability of LV to dilate, and consequently, increase SVI (via Frank-Starling mechanism) and progressively lose ability to increase HR (chronatropy). Thus, in survivors, LV dilatation may be an important compensatory mechanism for loss of chronatropic function.

With increasing dose of bacteria in survivors, there was also a temporal increase in LV size with maintenance of SVI and LVSWI (Figs. 3, 5, and 8*B*). This progressive increase in LV size in the surviving dog groups occurred without change in LV pressure, suggesting that LV became more compliant (Fig. 7). Further studies on septic shock are needed to determine the mechanism by which LV increases in size and maintains cardiac performance (SVI and SWI) without increasing LV pressure (PCWP) and development of pulmonary edema.

Nonsurvivors as compared with survivors in the highest dose group had a decrease in LV size, leftward shift of LV diastolic volume-pressure relationship, and greater decrease in CI, SVI, and SWI. In nonsurvivors, the leftward shift on LV diastolic volume-pressure plots was upward reflecting an increase in LV pressure (PCWP) (Fig. 7). A decrease in LV volume with an increase in LV pressure (PCWP) suggests a decrease in compliance (not intravascular volume loss). This compliance abnormality may have contributed to the poorer LV performance and possibly the demise of nonsurvivors. On LV function plots (Frank-Starling relationship plots and end-systolic volume-pressure plot), nonsurvivors compared with survivors (30×10^9) were downward shifted and not shifted as far to the right (Figs. 5 and 6). When comparing survivors and nonsurvivors, it is not possible to separate a circulating volume deficit from differences in LV systolic function as systemic pressure and preload are low. These decreases in LV size could be secondary to several possible mechanism: sepsis-induced changes in LV performance and compliance, more severe sepsis-induced decrease in intravascular volume, or both of these mechanisms. This more severe intravascular volume loss may represent a greater third space loss from peritoneal inflammation and/or a inadequate oral intake.

Recent reports on human septic shock indicate that survivors and nonsurvivors have a decreased LVEF; however, larger LV size is more common in survivors than in nonsurvivors (24, 25). Despite myocardial depression as evidenced by a decreased LVEF (24, 25, 27), septic human LV increases in size and maintains high or normal SVI and CI (7, 24). These data from the canine model further suggest that during septic shock, LV dilatation is a mechanism to increase or maintain SVI, SWI, and cardiac output despite systolic dysfunction.

Etiology and pathogenesis of these cardiac abnormalities during septic shock in humans and animals are

unknown (26). Recent studies in humans demonstrated that reduced coronary blood flow is an unlikely explanation (5). However, other recent investigations on human septic shock present evidence that a circulating myocardial depressant is associated temporally with decrease in EF (27, 28). The relationship of this circulating depressant to LV performance and compliance changes demonstrated in our study requires further investigation.

In conclusion, with an increasing dose of bacteria survivors of septic shock had progressive loss of systolic (inotropic and chronotropic) cardiac function. With increasing bacterial dose infected survivors progressively increased LV size without increasing pressure, i.e., shifting the volume-pressure relationship to the right. Despite increasing dose of bacteria, the survivors maintained similar levels of LV stroke volume and stroke work associated with survival. These data suggest that survivors with increasing bacterial dose are dependent on increases in LV size to maintain stroke volume and stroke work (Frank-Starling mechanism), and this LV dilatation may ultimately contribute to survival.

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